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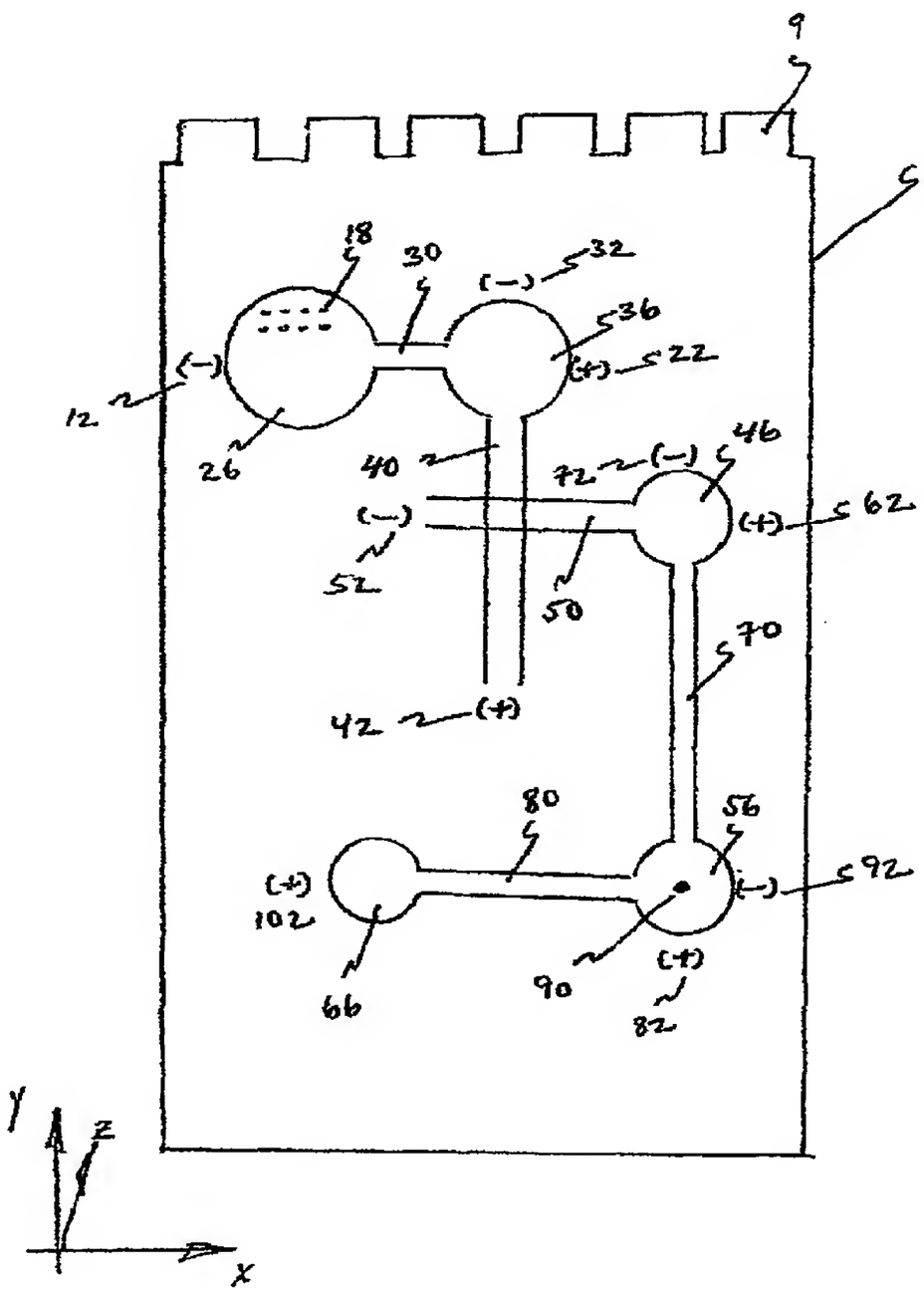
(54) Title: MICROFLUIDIC DEVICE FOR MOLECULAR ANALYSIS



(57) Abstract: An improved microfluidic device for the detection and analysis of desired chemical or biochemical components. The device allows molecular biological analysis and diagnosis using multistep reactions conducted on a small scale, with such reactions being conducted on or preferably within an electronic chip containing biochemical components that enable the detection of desired materials in an applied sample. Preferably, the device includes a sample chamber for lysis of sample cells using an applied AC field, a signal delivery chamber wherein signal delivery molecules are bound to desired target molecules of interest, and/or electrodes for detecting signal produced by the signal delivery molecules.

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Microfluidic Device for Molecular Analysis

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Related Applications

The present application claims the priority of U.S. Provisional Application Serial No. 60/309,221 filed August 1, 2001, which is fully incorporated herein by reference.

Field of the Invention

The present invention relates to the analysis of target solutions utilizing microelectromechanical systems, including microfluidic devices and methods.

Background of the Invention

The present invention relates to microelectromechanical systems (MEMS), and particularly to microfluidic devices for the analysis of biochemical or chemical components. Such devices, also known as biochips, have recently been developed in the art, and are discussed, for example, in U.S. Patent No. 5,849,486 issued December 15, 1998 to Heller et al.; U.S. Patent Application No. 6,127,125 issued October 3, 2000 to Yurino, et al.; U.S. Patent No. 6,174,683 issued January 16, 2001 to Hahn et al.; and U.S. Patent No. 6,197,503 issued to Vo-Dinh, et al.; all of which are fully incorporated herein by reference.

Examples and further background details of such devices have also have been disclosed by Caliper Technologies Corp. of Mountain View, California; by Aclara Biosciences, Inc. of Mountain View, California; by Cepheid of Sunnyvale, California; and by Gamera Bioscience Corp. formerly of Medford, Massachussetts (since acquired by Tecan Group Ltd. of Maennedorf, Switzerland)

among others.

Caliper's work in this area is disclosed in the references listed in Appendix A and in U.S. Patent Nos. 6,420,143; 6,416,642; 6,413,782; 6,413,401; 6,409,900; 6,406,905; 6,406,893; 6,399,389; 6,399,025; 6,399,023; 6,394,759; 6,391,622; 6,384,401; 6,379,974; 6,379,884; 6,366,924; 6,358,387; 6,353,475; 6,337,740; 6,337,212; 6,322,683; 6,321,791; 6,316,781;6,316,201; 6,306,659; 6,306,590; 6,303,343; 6,287,774; 6,287,520; 6,274,337; 6,274,089; 6,267,858; 6,251,343; 6,238,538; 6,235,471; 6,235,175; 6,233,048; 6,221,226; 6,186,660; 6,182,733; 6,174,675; 6,172,353; 6,171,850; 6,171,067; 6,167,910; 6,156,181; 6,153,073; 6,150,180; 6,150,119; 6,149,870;6,149,787; 6,148,508; 6,132,685;6,129,826; 6,123,798; 6,107,044; 6,100,541;6,090,251; 6,086,825; 6,086,740; 6,080,295; 6,074,725; 6,071,478; 6,068,752; 6,048,498; 6,046,056; 6,042,710; 6,042,709; 6,012,902; 6,011,252; 6,001,231; 5,989,402; 5,976,336; 5,972,187; 5,965,410; 5,965,001; 5,964,995; 5,959,291;5,958,694; 5,958,203; 5,975,579; 5,955,028; 5,948,227; 5,942,443; 5,885,470; 5,882,465;5,880.071; 5,876,675; 5,869,004; 5,852,495; 5,842,787; 5,800,690;5,779,868; and 5,699,157; all of which are fully incorporated herein by reference.

Aclara's work is disclosed in Appendix B and in and in U.S. Patent No. 6,399,952 (Multiplexed fluorescent detection in microfluidic devices); 6,344,326 (Microfluidic method for nucleic acid purification and processing); 6,322,980 (Single nucleotide detection using degradation of a fluorescent sequence; 6,306,273 (Methods and compositions for conducting processes in microfluidic devices; 6,284,113 (Apparatus and method for transferring liquids); 6,176,962 (Methods for fabricating enclosed microchannel structures); 6,103,537 (Capillary assays involving separation of free and bound species); 6,103,199 (Capillary electroflow apparatus and method); 6,093,296 (Method and device for moving molecules by the application of a plurality of electrical fields); 6,074,827 (Microfluidic method for nucleic acid purification and processing); 6,056,860

(Surface modified electrophoretic chambers); 6,054,034 (Acrylic microchannels and their use in electrophoretic applications); 6,043,036 (Method of sequencing nucleic acids by shift registering); 6,007,690 (Integrated microfluidic devices); 5,935,401 (Surface modified electrophoretic chambers); 5,883,211 (Thermoreversible hydrogels comprising linear copolymers and their use in electrophoresis); and 5,858,188 (Acrylic microchannels and their use in electrophoretic applications); all of which are fully incorporated herein by reference

Cepheid's work is disclosed in the references listed in Appendix C, and in U.S. Patent Nos. 6,403,037 (Reaction vessel and temperature control system); 6,374,684 (Fluid control and processing system); 6,369,893 (Multi-channel optical detection system); 6,368,871 (Non-planar microstructures for manipulation of fluid samples); 6,312,929 (Compositions and methods enabling a totally internally controlled amplification reaction); and 5,958,349 (Reaction vessel for heat-exchanging chemical processes); all of which are fully incorporated herein by reference.

Gamera's work is disclosed in 6,143,248 (Capillary microvalve) and 6,063,589 (Devices and methods for using centripetal acceleration to drive fluid movement on a microfluidics system); and 5,686,271 (Apparatus for performing magnetic cycle reaction), all of which are fully incorporated herein by reference.

Summary of the Invention

It is an object of the present invention to provide microfluidic devices for chemical analysis.

It is a further additional or alternative object of the present invention to provide an apparatus and method wherein a sample containing whole cells is added to a chamber in a biochip, the chamber being provided for preparation of the sample via pulsed field lysis / disruption of the cells using the application of suitable AC voltages.

It is a further additional or alternative object of the invention to provide an apparatus

having a chamber for lysis of whole cells using applied AC fields, and having a filter for separation of target molecules in the sample (e.g. from high molecular weight components and cell membrane fragments) after lysing of the sample.

It is a further additional or alternative object of the invention to provide an apparatus and method including the delivery of charged molecules from one chamber (or channel) of a biochip to further channels (or chambers) using applied electric fields. The applied fields are generated via sequential application of voltages at electrodes located in the appropriate biochip channels and/or chambers.

It is a further additional or alternative object of the invention to provide a biochip using electrophoretic processes to assist with sample analysis.

It is a further additional or alternative object to provide microfluidic devices having a signal delivery well preloaded with signal delivery molecules, the signal delivery molecules being molecules having a binding site with an affinity for a desired target molecule of interest, the signal delivery molecules further having a signalling component.

It is a further additional or alternative object to provide microfluidic devices having a signal delivery chamber preloaded with signal delivery molecules, the signal delivery molecules having a signalling component which producing an electrical signal.

It is a further additional or alternative object to provide microfluidic devices comprising a signal delivery chamber preloaded with signal delivery molecules, the signal delivery molecules having a binding site with an affinity for a desired target molecule of interest and having a signalling component, the signal delivery molecules being branched nucleic acids.

It is a further additional or alternative object to provide devices for chemical analysis, said devices having a chamber provided for detection of at least of a portion of a complex comprising a target molecule bound to a branched nucleic acid reagent.

It is a further additional or alternative object to provide devices for chemical analysis, said devices having branched nucleic acid signal delivery molecules, a chamber wherein binding occurs between said branched nucleic acid signal delivery molecules and target molecules of interest to form a bound complex of said signal delivery molecules and the target molecules; and an electrode for binding a component of said complex, and wherein said complex comprises a signalling component for generating an electric current which is detected at said electrode.

It is a further additional or alternative object of the invention to provide an apparatus and method including the use of a biochip having capture molecules at a working electrode for biochemical analysis.

It is a further additional or alternative object of the invention to provide an apparatus and method including the electrochemical detection of a target molecule at a working electrode using a suitable signal delivery molecule, the signal delivery molecule preferably being a branched nucleic acid (e.g. a 3DNA dendrimer) having the desired signal molecules linked thereto.

It is a further additional or alternative object of the invention to provide an apparatus and method including: a microfluidic device comprising a signal delivery chamber, the signal delivery chamber being preloaded with branched nucleic acid signal delivery molecules, the branched nucleic acid signal delivery molecules having a binding site with an affinity for a desired target molecule of interest and having a signalling component; and an electrode for binding a component of said complex, wherein said electrode detects an electric current generated by said signalling component.

Further objects and embodiments of the invention will become apparent from the detailed disclosure.

In accordance with the present invention, an improved microfluidic device is provided

herein for the detection and analysis of desired chemical or biochemical components. Such microfluidic devices allow molecular biological analysis and diagnosis using multistep reactions conducted on a small scale, with such reactions being conducted on or preferably within an electronic chip containing biochemical components that enable the detection of desired materials in an applied sample.

In the various embodiments of the invention, the desired biological or biochemical components provided in the biochip utilize suitable techniques from molecular biology in a sequence of predetermined processes or reactions that are guided or controlled by a driver/reader and the biochip's microelectronic circuitry. This circuitry preferably includes electronic contacts on the chip which are used for receiving and transmitting signals from the driver/reader and which also lead to electrodes located within the chip itself for initiating and controlling desired chemical movements and reactions. Specifically, internal chip electrodes provide desired AC and/or DC voltages and currents at specified locations within the chip in a predetermined sequence, causing the sequence of desired biochemical processes. After the sequence of desired processes or reactions has been completed, a signal is produced in the form of a resulting current which is output to a reader that interprets the resulting signal.

Consistent with the invention, the present systems can be used for medical use, environmental analyses, and so forth. In the preferred embodiment of the invention, the device is used for the detection and analysis of nucleic acids, with all purification, separation and detection steps occurring within the device. In a further preferred embodiment, the device is used for the detection and measurement of telomerase associated RNA and telomerase mRNA, which have been shown by Hexal to be valuable in the detection of cancer. *See e.g.*, PCT patent application publication numbers WO 97/18322, WO 99/40221, and WO 00/46585, all of whose disclosures are fully incorporated herein by reference.

In the preferred embodiment of the invention, the microfluidic device is provided with an initial sample area such as a chamber where whole cells are added, with the cells being osmotically lysed and/or electrically lysed on the chip itself, using pulsed field disruption of the cells in the well. Appropriate voltages are subsequently applied to the electrodes of the chip to establish electric fields between pair of electrodes, causing molecules of desired charge to be transported through the biochip's network of chambers and channels.

In this preferred embodiment, initially molecules of desired charge within the lysed sample in the sample chamber are transported through a size specific filter or plastic frit to a second chamber, a "staging" or origin well. At the staging well, suitably charged molecules within the crude analyte sample are transported through a channel provided for separation of target molecules by electrophoretic mobility. Molecules of the desired mobility are transferred through a detection channel to a chamber where the desired target molecules are bound to a predetermined signal delivery molecule to form a complex. The signal delivery molecule is preferably a branched nucleic acid which has been constructed by hybridization and/or cross-linking. Further preferably, the signal delivery molecules are dendrimer molecules (e.g. 3DNA^{TM}).

Preferably, the complex is then transferred through a further channel to an analysis chamber, where the target molecules are detected at an electrode. It is further preferred that the complex is delivered to bridge or bind with capture molecules linked to the working electrode (the detection electrode). Non-bound complexes and other waste material are transferred out of the analysis chamber to a waste chamber, leaving only bound target molecules in the analysis chamber. An electrochemical detection of those target molecules which have bound to the signal delivery molecules and bound to the working electrode is then conducted in the analysis chamber to detect an electrical signal output to a desired electronic device (such as a computer

reader). The presence of signal above background levels indicates the presence of target molecule in the sample of interest and its magnitude provides an indication of the relative concentration of target molecule in that initial sample.

Brief Description of the Drawings

Figure 1 is a schematic of a microfluidic biochip device in accordance with one preferred embodiment of the present invention.

Figure 2 is a schematic of a microfluidic biochip device in accordance with a further preferred embodiment.

Detailed Description of the Invention and the Preferred Embodiments

In accordance with the present invention, a biochip device is provided as shown, for example, in Figure 1. Biochip device 12 includes a series of sample wells and channels therein for enabling the detection and analysis of desired chemical components, and further includes a series of electrical contacts 9 connected to a driver/reader and extending into the biochip for providing and detecting appropriate voltages and currents at the wells and channels. For illustration purposes, the present application will discuss the use of the biochip in conjunction with the preferred embodiment, the detection and analysis of desired nucleic acids; however, it is to be understood that the principles of the invention can be applied to any desired chemical or biochemical structures, whether for medical use, environmental analyses, or so forth. In addition, whereas the present invention will generally discuss the sample device with a five well and five channel structure for illustration, it is to be understood that any additional number of wells and channels can easily be added to a desired biochip device consistent with the principles of the invention discussed herein. Likewise, although the application uses the terms chambers

(or wells) and channels, it will be understood that such terms are not meant to be limiting as processed discussed as conducted in a chamber can be conducted in a channel (or vice versa), with the terms further being intended to refer to any suitable areas, hollows, recesses or volumes, or so forth, of a microfluidic device.

The biochip itself is constructed of any material or surface suitable for the particular application, preferably plastic, glass or silicon. Alternatively, any other desired materials can be used consistent with the invention, provided that the material has a suitably low binding affinity to the reactions occurring on the internal surfaces of the chip.

The internal structure of the biochip is created by molding the chip of two separate halves, one or both of the halves having the desired channel and well structure. Preferably the bottom half is molded with the desired structure, and the top is provided as a flat coverslip thereon. During the molding process, suitable electrical contacts are inserted into or incorporated within the structure of the chip. For example, in the case of plastic, the chip is polymerized around the electrical circuitry.

Biochip device 12 includes a sample addition well 26 where whole sample cells or a sample lysate are added to the device for analysis. The sample can be added to the well 26 manually or via an automated system. Although a lysate can be added to sample well 26 if desired, the addition of whole cells is greatly preferred to simplify the use of the device, to eliminate unnecessary steps by the user or researcher, and to provide improved results. More generally, all purification, separation and detection steps on the sample are preferably conducted within the device, enabling a rapid analysis of a desired cellular sample in a manner which requires minimal labor and time. A preferred cell population to load into the sample well is telomerase positive circulating cancer cells prepared by the Hexal/Gentech Oncoquick™ device.

Upon addition of the whole cells to sample well or chamber 26, the cells are lysed within the well. Preferably, the cells are lysed using pulsed field sample cell disruption. Additionally or alternatively, the cells are added in an osmotically positive buffer relative to the cells. Alternately, in a non-preferred embodiment, the cells can be lysed using lysing reagents.

In the preferred embodiment, sample addition chamber 26 is approximately is 5ul to 500ul in size. In the y axis direction and/or z axis direction, the chamber 26 is provided with electrodes of a size comparable to the size of the sample chamber. In the figure, the x axis is defined as right to left, the y axis as bottom to top, and the z axis as normal to the page.

Sample disruption electrodes 18 are used to apply joule heating and disruption of the sample via an applied AC field. Application of the field results in sample cell membrane rupture, with the lysate, including any target nucleic acids, being emptied out into the sample chamber.

One example of a device configuration and method suitable for use as part of the present invention (including some suitable applied voltages and electric fields), is disclosed in S.W. Lee, H. Yowanto and Y.C. Tai, A Micro Cell Lysis Device, 1998, The 11th Annual International Workshop on Micro Electro Mechanical Systems (MEMS'98 Heidelberg, Germany), Jan. 1998, a copy of which is attached as Appendix E to U.S. Provisional Application Serial No. 60/309,221 filed August 1, 2001, and which is fully incorporated herein by reference. In the present method and device, the lysis can be conducted with or without sharpened electrodes such as disclosed in Lee.

Following disruption, a DC voltage is applied along the x-axis of sample well 26 to cause negatively charged molecules to migrate across the first chamber, sample well 26, and into and through a first channel, crude analyte collection channel 30. Specifically, an electric field is established between anode 12 in sample well 26 and cathode 22, causing negatively charged molecules in the lysate to migrate away from the anode of the sample well into the first channel

30 and toward the cathode in second chamber 36.

While Figure 1 illustrates the use of the biochip to detect target molecules of interest which are negatively charged (e.g. nucleic acids), in an alternate or additional embodiment, the biochip can likewise be used to detect positively charged molecules by reversing the polarity of the applied field. In various embodiments of the invention, the target molecule or substance can be any charged natural or artificial substance, including, but are not limited to, nucleic acids, proteins, peptide, carbohydrates, lipids, polysaccarides, glycoproteins, hormones, receptors, antigens, antibodies, viruses, pathogens, metabolic byproducts, growth factor, cofactors, intermediates, drugs, toxins, or so forth, providing the molecules carries or has been modified to carry, a charge thereon.

Upon application of the DC voltage and migration of the target molecules out of sample well 26, those molecules are directed into first collection channel 30. For example, voltages ranging from 1-10,000 v/cm can be applied. Collection channel 30 is a crude analyte collection channel used for the initial separation of the target molecules out of the lysate, separating those target molecules from fragments of cell membrane, cell debris, and other undesirable components. In one embodiment, the collection channel contains a plastic frit or other filter. This initial filter is preferably provided with a nominal porosity of 50nm to 200 microns, or of such porosity suitable for the intended application of the biochip.

Application of the electric field causes the crude analyte to further migrate out of collection channel 30 and into the second chamber of the biochip, staging well 36. The collection of crude analyte via applied voltage ensures that all of the molecules from the sample that arrive at the second chamber are negatively charged. Likewise, the use of a plastic frit or filter imposes a size limitation to begin the process of purifying out the target molecules from other, undesirable, molecules within the lysate (e.g., high molecular weight components and cell

membrane fragments), resulting in the delivery of a crude analyte to the second chamber having molecules of a desired charge and limited to a predetermined range of sizes.

In accordance with the invention, each of the anodes and cathodes of the biochip are electrodes provided in suitable locations in the chambers shown in Figure 1. Alternatively, or additionally, electrodes can be provided in the channels. In a further alternate embodiment, the electrodes can be located in each chamber or channel behind a frit which has a porosity smaller than the proposed analyte molecule. The electrodes can be separate from the chamber or channel, or portions of the chambers or channels can themselves be electrically charged to serve as the anodes and cathodes.

In a preferred embodiment, the electrodes are shaped and/or located to produce an electric field of a desired configuration to maximize the efficiency of molecular transfer. For example, in one embodiment, a parabolic electrode is provided for each anode and cathode so as to more effectively focus flux in the direction desired for migration of the target molecule.

Preferably, the analyte molecules are electrically focused to the middle of the second chamber prior to application of a voltage in the y-axis and migration of the molecules into the second channel, electrophoretic separation channel 40. Furthermore, in the preferred embodiment, the applied DC voltage that delivers the negatively charged molecules to the second chamber is of such duration and intensity to ensure that only relatively "small" molecules (i.e. RNA molecules), reach the crude second sample chamber 36, thereby excluding "large" molecules such as chromosomal nucleic acids (with small being typically up to approximately several 10,000 bases in the preferred embodiments, and usually less than approximately 5,000 bases, and large being typically greater than 1,000,000 bases).

Once the crude analyte sample has been isolated in chamber 36, voltages of desired intensity and duration are applied to anode 32 and cathode 42 to establish an electric field which

directs the target molecules into the second channel, electrophoretic separation channel 40. Channel 40 is designed to size fractionate the negatively charged molecules located in the crude analyte isolated in second chamber 36. Channel 40 is preferably filled with polyacrylamide (typically 4-12 w/v%) for electrophoresis. The behavior of channel 40 is utilized to fractionate the negatively charged molecules based on electrophoretic mobility. Thus, the device uses a first "cut" based primarily on charge and crudely on size, and uses a second separation based primarily on electrophoretic mobility, the device being blocked at one end thereby preventing electrokinetic flow.

As shown in Figure 1, a delivery or detection channel 50 is located at the location where the target molecule is electrophoretically focused, the channel 50 being provided for subsequent transport of the desired target molecule. In a further embodiment, the channel can be provided with a microvalve at the entrance to the fourth channel 50. Following the appropriate electrophoresis in separation channel 40 (appropriate being defined such that the center of the concentration gradient of analyte molecules is centered on the delivery channel 50), the y-axis field within channel 40 resulting from the voltages at electrodes 32 and 42 ceases. Voltages are then applied at electrodes 52 and 62 to establish an electric field which migrates the target molecule into and through detection channel 50. In a manner comparable to the initial collection of negatively charged molecules, the target analyte molecules (typically RNA), migrate to a third chamber 46, the hybrization well (also referred to herein as the signal delivery well)

Hybridization or signal delivery well 46 is a chamber provided with a relatively large concentration of signal delivery molecules to facilitate the detection of the desired target molecules. The signal delivery molecules are molecules having a binding area with an affinity for the desired target molecule of interest, and having a signalling component, the signalling component preferably producing an electrical current. Further preferably, the signal delivery

molecules are branched nucleic acids, the nucleic acids being branched by hybridization and/or cross-linking.

In the preferred embodiment, the signal delivery molecules are dendrimers (e.g. 3DNA) which have been designed to be specific for the target (nominal 1ng/ul typical volume 5-50ul), or other hyperbranched molecules or matrices of nucleic acids. Dendrimer technology is disclosed in U.S. Patent Nos. 5,175,270; 5,484,904; 5,487,973; 6,072,043; 6,110,687; 6,117,631; in Nilsen et al., Dendritic Nucleic Acid Structures, J. Theor. Biol., 187, 273-284 (1997); in Stears et al., A Novel, Sensitive Detection System for High-Density Microarrays Using Dendrimer Technology, Physiol. Genomics, 3: 93-99 (2000); PCT Application Serial No. PCT/US01/07477; and published protocols available from Genisphere, Inc. of Montvale, New Jersey; all of those disclosures being fully incorporated herein by reference.

The dendrimer molecule specificity is conferred via any analyte-recognizing-moiety bound to the dendrimer molecule – for RNA (nucleic acid targets) the specificity is preferably DNA, RNA, PNA, LNA or any polymer that specifically recognizes via base pairing with the target RNA molecule.

The signal delivery molecules are preferably predispensed into the third chamber prior to providing the biochip to the user, e.g. via a septum located at the top of chamber 46. In further alternative or additional embodiments, appropriate microvalves are provided at the entrance and exit of chamber 46 to isolate the signal delivery molecules in the third chamber during transport of the biochip. Alternatively, the signal delivery molecule can be dispensed into chamber 46 by the user or by an automated device upon delivery of the target molecules into that chamber.

In the hybridization or signal delivery well 46, the target molecules delivered through detection channel 50 bind to the predetermined signal delivery molecules (e.g. dendrimer molecules) located in the well. The signal delivery molecule (preferably a branched nucleic acid

or a hyperbranched nucleic acid such as a dendrimer) contain the signalling component(s) which will be later used to generate a signal in a subsequent chamber. In the preferred embodiment, the signal is an electrical current generated at the working electrode (the detection electrode).

In one preferred embodiment, the signalling component or signal molecule of the signal delivery molecules are the "G" residues of a 3DNA molecule, as previously disclosed by the H. Holden Thorp and colleagues at the University of North Carolina at Chapel Hill, and by Xanthon, Inc. of Research Triangle Park, North Carolina (*see e.g.*, U.S. Patent Nos 6,180,346; 6,132,971; 6,127,127; 5,968,745; 5,871,918; and 5,171,853; all of which are fully incorporated herein by reference). Use of the G residues is preferred; alternatively, the signal residues can be another preselected base (e.g., adenine, 6-mercaptoguanine, 8-oxo-guanine, or 8-oxo-adenine). As discussed by Thorp et al., the nucleic acid is reacted with a transition metal complex capable of oxidizing the preselected base in an oxidation-reduction reaction, and the oxidation-reduction reaction is detected to determine the presence or absence of the nucleic acid from the detected oxidation-reduction reaction at the preselected base.

In an further alternate or additional embodiment, the signal molecules are electron donor molecules bound to 3DNA molecules. Examples of suitable electron donors include the rare earth cryptands and caged neutral metal atoms, and coordinated metals that can be further oxidized. Preferably, a preselected base and electron donor molecules are both utilized for signalling.

Upon delivery of the target molecule to chamber 46, the target molecules bind to the signal delivery molecules in this chamber, preferably by hybridization. Following binding (preferably at room temperature), voltages are applied in the y-axis to electrodes 72 and 82 to transport the signal delivery molecule with bound analyte through channel 70 into the fourth chamber, the analysis chamber or working electrode well 56.

In analysis chamber 56, a working electrode 90 (the "detection electrode") is provided which will detect the target molecule. Preferably, a portion of the complex of target molecule and signal delivery molecule will bind to the working electrode. Further preferably, a working electrode is provided which has been modified with capture molecules that will bind the target molecule (or alternatively that will bind the signal delivery molecule), the binding preferably being by hybridization. In the preferred embodiment, neutral capture molecules are utilized. However, in an alternate embodiment, DNA oligonucleotides can be used at the working electrode as demonstrated by Thorp, et al.

In the preferred embodiment, capture molecules are provided which bind to a site on each target molecule which is different from the target's binding site for the signal delivery molecule. In other words, it is preferred that the target molecule serve as a bridge between the signal delivery molecule and the working electrode 90. In addition, the molecules bound to the working electrode that serve to capture the target molecule are electrochemically neutral.

In the preferred embodiment and for nucleic acid (RNA) targets, the capture molecules are preferably composed of PNA, DNA, RNA, LNA or any polymer that specifically recognizes via base pairing with the target RNA molecule. When the signal molecules are the G residues of 3DNA, these capture molecules lack the "G" base so as not to contribute to the subsequent electrochemical detection at the working electrode.

Following "bridging" of some or all of the signal delivery molecules molecules to the working electrode, a voltage is applied in the x axis to electrodes 92 and 102 to cause unbound signal delivery molecules and negatively charged molecules to migrate through channel 80 to the fifth chamber, waste well 66. In the preferred embodiment, the applied electric field in the x-axis can be tuned to the strength of the interaction between the target molecule and signal delivery molecule and target molecule and the working electrode. In other words an applied

electric stringency is used. By "stringency", the present application refers to the ability to discriminate between specific and non-specific binding interactions by changing a physical parameter. Although stringency can be regulated using temperature control for the nucleic acid hybridizations of the present invention, preferably an electric stringency is utilized. The concept of stringency via applied electric field has been used extensively by Nanogen (*see e.g.*, U.S. Patent Nos. 6,180,346; 6,132,971; 6,127,127; 5,968,745; 5,871,918; 6,245,508; 6,238,869; 6,238,624; 6,232,066; 6,225,059; 6,207,373; 6,197,503; 6,162,603; 6,129,828; 6,099,803; 6,071,394; 6,068,818; 6,054,277; 6,051,380; 6,017,696; 6,013,166; 5,965,452; 5,849,489; 5,849,486; 5,728,532; 5,632,957; 5,605,662; and, 5,565,322; whose disclosures are fully incorporated herein by reference).

Once migration of the unbound signal delivery molecules molecules and any other negatively charged waste to the waste well 66 has been completed, the target molecules of interest have been effectively isolated in the analysis chamber 56 and bound to the working electrode. In the analysis chamber, an electric field is then applied to the working electrode 90 and a current is provided by the signal molecules on the signal delivery molecules (e.g. 3DNA dendrimers) which is measured by the reader connected at electrical contacts 9. Preferred electrodes for the electrochemical detection of the target molecules (when the molecules are nucleic acids) are indium doped tin oxide modified with 4-vinyl-4'-methyl-2,2'-bipyridine or comparable compounds as detailed in "Electrochemical Detection of Single-Stranded DNA Using Polymer-Modified Electrodes" *Inorg. Chem.*, 1999, 38 1842-1846, the disclosure of which is fully incorporated herein by reference.

The total amount of current generated at the working electrode is then measured by the reader and provided on a digital readout. The presence of this current (if any) indicates the presence of the target molecule in the original sample. If a current is measured over the current

produced (if any) by a no-target control, that excess current over the control current is proportional to the amount of target molecule in the original sample.

Although a variety of preferred techniques are provided herein, it is to be understood that numerous variations are possible consistent with the invention. For example, in addition to, or in place of, nucleic acid hybrization analysis, antibody/antigen analysis can be used or so forth as desired.

Likewise, in a further embodiment of the invention, more than one target molecule can be isolated using separation channel 40. As shown in Figure 1, in a first embodiment as described above, desired target molecules are subsequently migrated through a delivery or detection channel 50. In an further embodiment, as shown in Figure 2, additional target molecules can also be isolated, based on their different rates of migration through the electrical field. Thus, a second group of molecules can be isolated and delivered through a second delivery channel 150, a third group of molecules isolated and delivered through a third delivery channel 250, and so forth, with as many delivery channels being provided as desired. For each of the additional target molecules and delivery channels 150, 250, a set of third chambers 146, 246, fourth chambers 156, 256, and fifth chambers 166, 266 (and associated channels) are provided in a manner analogous to the network of channels and chambers used for the first target molecules passing through the detection channel 50.

Similarly, while a single type of charged molecule has been discussed (e.g. a negatively charged molecule in the case of RNA), in a further alternate or additional embodiment of the invention, the biochip can be used to simultaneously separate both negatively and positively charged molecules. In this embodiment, for example, a second network of chambers is provided to the left of sample well 26, this second network being a mirror image of the chambers and channels provided in Figure 1 on the sample well's right side. The network provided on the left

of the first chamber operates using electrodes and voltages of opposite polarities to those provided on the right. Thus, negatively charged molecules migrate through a network on the right side of the chip, and positively charged molecules migrate through a network on the biochip's left (or vice versa).

Having described the present inventions with regard to specific embodiments, it is to be understood that the description is not meant as a limitation since further embodiments, modifications and variations may be apparent or may suggest themselves to those skilled in the art. It is intended that the present application cover all such embodiments, modifications and variations.

Appendices

The following references, which provide further background information to the present invention, are all fully incorporated herein by reference.

Appendix A: Caliper References

Ramsey, J. Michael et al., Microfabricated chemical measurement systems, Nature Medicine, 1(10):1093-1096, 10/1995.

Kopf-Sill, Anne R. et al., Complexity and performance of on-chip biochemical assays, SPIE Proceedings of Micro- and Nanofabricated Electro-Optical Mechanical Systems for Biomedical and Environmental Applications, 2978:172-179, 2/10/97-2/11/97.

Bousse, Luc J. et al., An Electrophoretic Serial to Parallel Converter, Transducers 97 (Chicago): 1997 International Conference on Solid-State Sensors and Actuators, 1:499-502, 6/16/97-6/19/97.

Bousse, Luc J. et al., Parallelism in integrated fluid circuits, SPIE Proceedings of Systems and Technologies for Clinical Diagnostics and Drug Discovery, 3259:179-186, 1/26/98-1/27/98.

Bousse, Luc J. et al., High-Performance DNA Separations in Microchip Electrophoresis Systems, Micro Total Analysis Systems '98: Proceedings of the uTAS '98 Workshop, 271-275, 10/13/98-10/16/98.

Bousse, Luc J. et al., Optimization of Sample Injection Components in Electrokinetic Microfluidic Systems, IEEE International MEMS '99, 309-314, 1/17/99-1/21/99.

Cohen, Claudia B. et al., A Microchip-Based Enzyme Assay for Protein Kinase A, Analytical Biochemistryv, 273:89-97, 8/1999.

Bousse, Luc J., Electrokinetic Microfluidic Systems, SPIE Microfluidic Devices and Systems II, 3877:2-8, 9/20/99-9/21/99.

Lazar, Iulia M. et al., Subattolmole-Sensitivity Microchip Nanoelectrospray Source with time-of-flight Mass Spectrometry Detection, Anal. Chem., 71:3627-3631, 9/1/99.

Nikiforov, Theo T. et al., Detection of Hybrid Formation between Peptide Nucleic Acids and DNA by Fluorescence Polarization in the Presence of Polylysine, Analytical Biochemistry, 275:248-253, 11/15/1999.

Jeong, Sang et al., Kinase Assay Based on Thiophosphorylation and Biotinylation, BioTechniques, 27:1232-1238, 12/1999.

Sundberg, Steven A., High-throughput and ultra-high-throughput screening: solution- and cell-based approaches, Current Opin in Biotech., 11:47-53, 11:47-53.

Coffin, Jill et al., Detection of phosphopeptides by fluorescence polarization in the presence of cationic polyamino acids: Application to kinase assays, Anal. Biochem., 278:206-212, 2/2000.

Knapp, Michael R. et al., Test Tube's End, J. Biomol. Screening, 5(1):9-12, 2/2000.

Bousse, Luc J. et al., Electrokinetically Controlled Microfluidic Analysis Systems, Annu. Rev. Biophys. Biomol. Struct., 29:155-181, 7/2000.

Sundberg, Steven A. et al., Microchip-based systems for target validation and HTS, Drug Discovery Today, 5(12) Supp:92-103, 12/2000.

Rocklin, Roy D. et al., A microfabricated fluidic device for performing two-dimensional liquid-phase separations, Anal. Chem., 72:5244-5249, 11/2000.

Nikiforov, T.T., New applications of fluorescence polarization for enzyme assays and in genomics, SPIE, 4255:94-105, 01/2001.

Hodge, C.N. et al., Microfluidic analysis, screening, and synthesis, High-Throughput Synthesis: Principles and Practices, Chapter 9, pp. 303-330, 02/2001.

Bousse, L.J. et al., Protein sizing on a microchip, Anal. Chem., 73:1207-1212, 3/2001.

Chien, Ring-Ling et al., Multiport flow-control system for lab-on-a-chip microfluidic devices, J. Anal. Chem., 371:106-111, 07/2001.

Yang, Hua et al., Sample stacking in laboratory-on-a-chip devices, J. Chrom., 924:155-163, 10/2001.

Appendix B: Aclara References

TD Boone, AJ Ricco, ZH Fan, HD Tan, SJ Williams, HH Hooper, (2002) Analytical Chemistry. Plastic Advances Microfluidic Devices. Volume 74, Issue 3: 78A - 86A.

Cronin, MT, ES Mansfield, (2001) Microfluidic Arrays in Genetic Analysis. Expert Review of Molecular Diagnostics. 1(1): 109-118.

Cronin, MT, M Pho, D Dutta, F Frueh, L Schwarcz, and T Brennan (2001) Utilization of New Technologies in Drug Trials and Discovery. Drug Metabolism and Disposition, 29(4): 586-590.

Cronin, MT, T Boone, AP Sassi, H Tan, Q Xue, SJ Williams, AJ Ricco and HH Hooper (2001)

Plastic Microfluidic Systems for High Throughput Genomic Analysis and Drug Screening. Journal of the Association for Laboratory Automation. 6(1): 74-78.

Travis Boone, Antonio Ricco, Torleif Bjornson, et al: "High-Throughput Drug Discovery and Genetic Analysis on Plastic Microfluidic Devices," Abstract from MipTec-ICAR: 3rd International Conference on Microplate Technology, Laboratory Automation and Robotics, Oct 2000, Basel, CH.

Alexander P. Sassi, Qifeng Xue, and Herbert H. Hooper (2000)Making Analysis in the Life Sciences Faster Through Miniaturization. American Laboratory. pp.36-41.

2000 Alexander P. Sassi, Aran Paulus, Ingrid D. Cruzado, Tor Bjornson, Herbert H. Hooper (2000). ìiRapid, parallel separations of D1S80 alleles in a plastic microchannel chipîî, Journal of Chromatography A, 894, pp. 203-217.

Weber, W.W. and Cronin, M.T., (2000), Pharmacogenetic Testing, in Encyclopedia of Analytical Chemistry, R.A. Meyers, Ed., John Wiley and Sons, Ltd., Sussex. pp. 1506-1531.

Ian Gibbons (2000) Microfluidic Arrays for High-throughput Submicroliter Assays Using Capillary Electrophoresis. Drug Discovery Today: HTS supplement. 1(1): 33-37.

Ian Gibbons (2000) Microfluidic Arrays for High-throughput Submicroliter Assays Using Capillary Electrophoresis. Drug Discovery Today: HTS supplement. Volume 1, Issue 1, pp. 33-37.

Travis Boone, Antonio Ricco, Philip Gooding, et al: "Sub-Microliter Assays and DNA Analysis on Plastic Microfluidics," Proceedings of the uTAS 2000 Symposium, Enschede, Netherlands, May 2000, pp.541-544.

Laviero Mancinelli, Maureen Cronin, Wolfgang Sadéée. Pharmocogenomics: The Promise of Personalized Medicine. AAPS Pharmsci 2000; 2 (1) article 4

Alexander Sassi, Tony Ricco, Herbert Hooper, "For Electrophoresis, Smaller is Faster," Todays Chemist at Work, Vol. 9, No. 2, Feb. 2000, pg. 44.

Abstract from Society for Biomolecular Screening 5th Annual Meeting, "Screening of Enzyme Inhibition by Capillary Electrophoresis in Single-Use LabCardTM Microfluidic Devices"

Rapid and Sensitive Chip-CE Method for High-Throughput Screening of Kinase Inhibitors
Qifeng Xue, Ann Wainright, Thu Trong Le and Ian Gibbons
Poster at High Performance Capillary Electorphoresis Meeting, Feb 99. Palm Springs CA

Nanyan Zhang, Hongdong Tan, and Edward S. Yeung* Automated and Integrated System for High-Throughput DNA Genotyping Directly from Blood, Analytical Chemistry; 1999; 71(6); 1138-1145.

Yonghua Zhang, Hongdong Tan, and Edward S. Yeung* ; Multiplexed Automated DNA Sequencing Directly from Single Bacterial Colonies, Analytical Chemistry; 1999; 71(22); 5018-5025.

Travis D. Boone, Herbert H. Hooper, and Cahners Business Information: "Industry Profile: ACLARA BioSciences concentrates on biomicrosystems development," Micromachine Devices, Vol. 3 No. 7, July 1998, pp. 1,4-6.

Travis D. Boone, Herbert H. Hooper, and David S. Soane: "Integrated Chemical Analysis on Plastic Microfluidic Devices," Solid State Sensor and Actuator Workshop Proceedings, Hilton Head Island, South Carolina(1998), pp. 87-92.

Angelika Muscate, Francois Natt, Aran Paulus, and Markus Ehrat: "Capillary Affinity Gel Electrophoresis for Combined Size- and Sequence-Dependent Separation of Oligonucleotides," Anal. Chem 70 (1998)

Hongdong Tan and Edward S. Yeung* ; Automation and Integration of Multiplexed On-Line Sample Preparation with Capillary Electrophoresis for High-Throughput DNA Sequencing, Analytical Chemistry; 1998; 70(19); 4044-4053.

Paulus, A., S.J. Williams, A.P. Sassi, P. Kao, H. Tan, and H. H. Hooper: "Integrated Capillary Electrophoresis using Glass and Plastic Chips for Multiplexed DNA Analysis," SPIE Proceedings 3515 94-103 (1998).

S. Attiya, X.C.Qiu, G. Ocvick, N. Chiem, W.Lee, D.J. Harrison: "Integrated Microsystem for Sample Introduction, Mixing, Reaction, Separation and Self Calibration of Immunoassays," Proceedings of the μμ-TAS'98 Workshop, Banff, Alberta, CANADA, pp.231

Aran Paulus, Stephen J. Williams, Alexander P. Sassi, Pin Kao, Hongdong Tan and Herbert Hooper: "Integrated Capillary Electrophoresis using Glass and Plastic Chips for Multiplexed DNA Analysis,"(1998) SPIE Vol. 3515, Microfluidic Devices and Systems, 94-103.

Carlo S. Effenhauser, Gerard J.M. Bruin, and Aran Paulus: "Integrated Chip-Based Capillary Electrophoresis - A Review," Electrophoresis 18 (1997) 2203-2213.

Carlo S. Effenhauser, Gerard J.M. Bruin, Aran Paulus, and Markus Ehrat: "Integrated Capillary Electrophoresis on Flexile Silicon Microdevices: Analysis of DNA Restriction Fragments and Detection of Single DNA Molecules on Microchips," Anal. Chem. 69 (1997) 3451-3457.

Randy M. McCormick, Robert J. Nelson, M. Goretty Alonso-Amigo, Dominic J. Benvegnu, and Herbert H. Hooper: "Microchannel Electrophoretic Separations of DNA in Injection-Molded Plastic Substrates," Anal. Chem. 69 (1997) 2626-2630.

Hongdong Tan and Edward S. Yeung*; Integrated On-Line System for DNA Sequencing by Capillary Electrophoresis: From Template to Called Bases, Analytical Chemistry; 1997; 69(4); 664-674.

Hooper, H. H., J. Yu, A. P. Sassi, and D. S. Soane: "Viscosity Transitions in Aqueous Suspensions of Hydrogel Microspheres," Journal of Applied Polymer Science, 63 (10), 1369 (1997).

May 1996 Aran Paulus: "Separation, Characterization, and Fraction Collection in the Nanoliter Domain with Capillary Electrophoresis," Angew. Chem. Int. Ed. 35 (1996) 857-859.

Sassi, A.P., A. Barron, M. G. Alonso-Amigo, D. Y. Hion, J. S. Yu, D. S. Soane, and H. H. Hooper: "Electrophoresis of DNA in Novel Thermoreversible Matrices," Electrophoresis, 17, 1460 (1996).

Andreas Manz, D. Jed Harrison, Elisabeth M.J. Verpoorte, James C. Fettinger, Aran Paulus, Hans LŸŸdi, and H. Michael Widmer: "Planar Chip Technology for Miniaturisation and Integration of Separation Techniques into Monitoring Systems: Capillary Electrophoresis on a Chip," J. Chromatogr. 593 (1992) 253-258.

Appendix C: Cepheid References

MT Taylor, GTA Kovacs, P Belgrader, R Joshi, S Sakai, MA Northrup, and KE Petersen.

""Disrupting Bacterial Spores and Cells using Ultrasound Applied through a Solid Interface."" 2nd IEEE-EMBS Conf. on Microtech. in Med. & Bio. 551-555, 2002.

M Jones, D Alland, M Marras, H El-Hajj, MT Taylor, and W McMillan. ""Rapid and Sensitive Detection of Mycobacterium DNA using Cepheid SmartCycler and Tube Lysis System."" Clin. Chem. 47(10): 1917-1918, 2001.

MT Taylor, P Belgrader, R Joshi, GA Kintz, and MA Northrup. ""Fully Automated Sample Preparation for Pathogen Detection Performed in a Microfluidic Cassette."" Micro Total Analysis Systems, 670-672, 2001.

MT Taylor, F Raisi, P Belgrader, F Pourahmadi, AE Herr, GA Kintz, and MA Northrup. ""Microfluidic Bioanalysis Cartridge with Interchangeable Mirochannel Separation Components."" Transducers '01 Eurosensors XV, Munich, 1214-1217, 2001.

F Raisi, P Belgrader, DA Borkholder, AE Herr, GJ Kintz, F Pourhamadi, MT Taylor, and MA Northrup. ""Microchip Isoelectric Focusing Using a Miniature Scanning Detection System."" Electrophoresis, in press, 2001.

P Belgrader, S Young, B Yuan, M Primeau, LA Christel, F Pourahmadi, and MA Northrup. ""A Battery-Powered Notebook Thermal Cycler for Rapid Multiplex Real-Time PCR Analysis."" Analytical Chemistry, 73, 286-289, 2001.

MT Taylor, P Belgrader, BJ Furman, F Pourahmadi, GA Kovacs, and MA Northrup. ""Lysing Bacterial Spores by Sonication Through a Flexible Interface in a Microfluidic System."" Analytical Chemistry, 73(3), 492-496, 2001.

F Pourahmadi, M Taylor, GA Kovacs, K Lloyd, S Sakai, T Schafer, B Helton, L Western, S Zaner, J Ching, WA McMillan, P Belgrader, and MA Northrup. ""Toward a rapid, integrated, and fully automated DNA diagnostic assay for Chlamydia trachomatis and Neisseria gonorrhoeae."" Clinical Chemistry, 46(9):1511-3, 2000.

F Pourahmadi, K Lloyd, P Belgrader, G Kovacs, R Chang, M Taylor, S Sakai, T Schafer, B McMillan, K Petersen and MA Northrup. ""Versatile, adaptable and Programmable Microfluidic Platforms for DNA diagnostics and Drug Discovery Assays."" Micro Total Analysis Systems (μμTAS) 2000, 243-248, May 2000.

P Belgrader, M Okuzumi, F Pourahmadi, D Borkholder, and MA Northrup. ""A microfludic cartridge to prepare spores for PCR analysis."" Biosensors and Bioelectronics, 14, 849-852, 2000.

P Belgrader, D Hansford, GA Kovacs, K Venkateswaren, F Milanovich, S Nasarabadi, R Mariella, M Okuzumi, F Pourahmadi, and MA Northrup. ""A minisonicator to rapidly disrupt bacterial spores."" Analytical Chemistry, 71, 4232-4236, 1999.

S Nasarabadi, F Milanovich, J Richards, and P Belgrader. ""Simultaneous Detection of TaqMan®® probes containing FAM and TAMRA Reporter Fluorophores."" Biotechniques, 27, 1116-1118, 1999.

P Belgrader, W Benett, D Hadley, J Richards, P Statton, R Mariella Jr., and F Milanovich. ""Detection and identification of bacteria in 7 minutes using a portable PCR instrument."" Science, 284, 449-450, 1999.

MA Northrup, L Christel, WA McMillan, K Petersen, F Pourahmadi, LWestern, and S Young. ""A New Generation of PCR Instruments and Nucleic Acid Concentration Systems."" PCR

Applications - Protocols for Functional Genomics, 105-125, 1999.

P Belgrader, W Benett, D Hadley, G Long, R Mariella Jr., F Milanovich, S Nasarabadi, W Nelson, J Richards, and P Stratton. ""Rapid Pathogen Detection Using a Microchip PCR Array Instrument."" Clinical Chemistry, 44, 2191-2194, 1998.

K Petersen, WA McMillan, GA Kovacs, MA Northrup, L Christel, and F Pourahmadi. ""The Promise of Miniaturized Clinical Diagnostic Systems."" IVD Technology, 4(4), 43-49, 1998.

MA Northrup, L Christel, WA McMillan, K Petersen, F Pourahmadi, LWestern, and S Young. ""A New Generation of PCR Instruments and Nucleic Acid Concentration Systems,"" in PCR Protocols, Innis, Gelfand, and Sninsky (eds), Academic Press, San Diego, Chapter 8, 1998.

LA Christel, K Petersen, WA McMIllan, and MA Northrup. ""Rapid, Automated Nucleic Acid Probe Assys Using Silicon Microstructures for Nucleic Acid Concentration."" Journal of Biomedical Engineering, 121, 22-25, 1998.

MS Ibrahim, RS Lofts, EA Henchal, P Jahrling, VW Weedn, MA Northrup, and P Belgrader. ""Real-time Microchip PCR for Detecting Single-Base Differences in Viral and Human DNA."" Analytical Chemistry, 70(9), 2013-2017, 1998.

MA Northrup, D Hadley, P Landre, S Lehew, J Richards, and P Stratton. ""A Miniature DNA-based Analytical Instrument Based on Micromachined Silicon Reaction Chambers."" Analytical Chemistry, 70 (5), 918-922, 1998.

P Belgrader, JK Smith,VW Weedn, and MA Northrup. ""Rapid PCR for Identity Testing Using a Battery-Powered Miniature Thermal Cycler."" Journal of Forensic Science, 43 (3), 315-319, 1998.

MA Northrup, B Beeman, D Hadley, P Landre, and S Lehew; in Automation Technologies for Genome Characterization, T. J. Beugelsdijk (ed), J Wiley & Sons, New York, Chapter 9, 1997.

AT Woolley, D Hadley, P Landre, AJ deMello, RA Mathies, and MA Northrup. "Functional Integration of PCR Amplification and Capillary Electrophoresis in a Microfabricated DNA Analysis Device." Analytical Chemistry, 68, 4081-86, 1996.

MA Northrup, B Beeman, D Hadley, P Landre, and S Lehew. Analytical Methods and Instrumentation, Special Issue on MicroTAS, H. M. Widmer (ed) c/o Ciba Geigy, Basel, 153-157, 1996.

Claims

What is claimed is:

1. A device for chemical analysis, comprising:

a microfluidic device comprising a sample chamber for preparation of a sample, wherein the sample is lysed in said sample chamber using an AC field applied to the sample; and,

a filter for separation of target molecules from high molecular weight components in the sample after lysing of the sample.

2. A device as claimed in claim 1, wherein said filter separates out said target molecules on the basis of size.

3. A device as claimed in claim 1, wherein the target molecules are transferred from said sample chamber through said filter using an electrical field.

4. A device for chemical analysis, comprising:

a microfluidic device comprising a signal delivery well, said signal delivery well being preloaded with signal delivery molecules, said signal delivery molecules being molecules having a binding site with an affinity for a desired target molecule of interest and having a signalling component.

5. A device as claimed in claim 4, wherein said signalling component produces an electrical current detectable by said device.

6. A device as claimed in claim 4, wherein said signalling component is at least one G residue of a branched nucleic acid molecule.

7. A device as claimed in claim 4, wherein said signalling component is an electron donor molecule bound to a branched nucleic acid molecule.

8. A device for chemical analysis, comprising:

a microfluidic device comprising a signal delivery chamber, said signal delivery chamber being preloaded with signal delivery molecules, said signal delivery molecules having a binding site with an affinity for a desired target molecule of interest and having a signalling component; and,

wherein said signal delivery molecules comprise branched nucleic acids.

9. A device as claimed in claim 8, wherein said branched nucleic acids are dendrimers.

10. A device as claimed in claim 8, wherein said signalling component produces an electrical current.

11. A device as claimed in claim 8, wherein said signalling component is at least one G residue of a branched nucleic acid molecule.

12. A device as claimed in claim 8, wherein said signalling component is an electron donor molecule bound to a branched nucleic acid molecule.

13. A device for chemical analysis, comprising:

a microfluidic device for biochemical analysis, said device having a chamber for detection of at least of a portion of a complex comprising a target molecule bound to a branched nucleic acid molecule.

14. A device as claimed in claim 13, wherein said chamber comprises an electrode for

detecting an electrical signal produced by a component of the complex of a target molecule bound to said branched nucleic acid molecule.

15. A device as claimed in claim 13, wherein said branched nucleic acid is a dendrimer.

16. A device as claimed in claim 13, wherein a signal produced by at least one G residue of said branched nucleic acid molecule is detected in said chamber.

17. A device as claimed in claim 13, wherein a signal produced by an electron donor molecule bound to said branched nucleic acid molecule is detected in said chamber.

18. A device for chemical analysis, comprising:

(a) a microfluidic device comprising branched nucleic acid signal delivery molecules;

(b) a chamber wherein binding occurs between said branched nucleic acid signal delivery molecules and target molecules of interest to form a bound complex of said signal delivery molecules and the target molecules; and,

(c) an electrode for binding a component of said complex, and wherein said complex comprises a signalling component for generating an electric current which is detected at said electrode.

19. A device as claimed in claim 18, wherein said electrode comprises a capture molecule for binding a component of said complex.

20. A device as claimed in claim 19, wherein said capture molecule binds to the target molecule.

21. A device as claimed in claim 20, wherein said capture molecule binds to the target molecule at a different site from the binding of the target molecule to the signal delivery molecule.

22. A device as claimed in claim 21, wherein said capture molecule does not produce a signal detectable by said electrode.

23. A device as claimed in claim 18, wherein the target molecule acts as a bridge between said signal delivery molecule and said electrode.

24. A device as claimed in claim 18, wherein said signal delivery molecule comprises said signalling component.

25. A device as claimed in claim 18, wherein said signal delivery molecule is a dendrimer.

26. A device as claimed in claim 18, wherein said signalling component is a G residue.

27. A device as claimed in claim 18, wherein said signalling component is an electron donor molecule.

28. A device for chemical analysis, comprising:

(a) a microfluidic device comprising a signal delivery chamber, said signal delivery chamber being preloaded with branched nucleic acid signal delivery molecules, said branched nucleic acid signal delivery molecules having a binding site with an affinity for a desired target molecule of interest and having a signalling component; and,

(b) an electrode for binding a component of said complex, wherein said electrode detects an electric current generated by said signalling component.

29. A device as claimed in claim 28, wherein said device comprises a sample chamber for preparation of a sample, wherein the sample is lysed in said sample chamber using an AC field applied to the sample.

30. A device as claimed in claim 29, wherein said device comprises a filter to separate target molecules in the sample from cell membrane fragments after lysing of the sample.

31. A device as claimed in any of claims 28-30, wherein said device comprises channels for electrophoretic separation of the target molecules from the sample.

FIG. 1

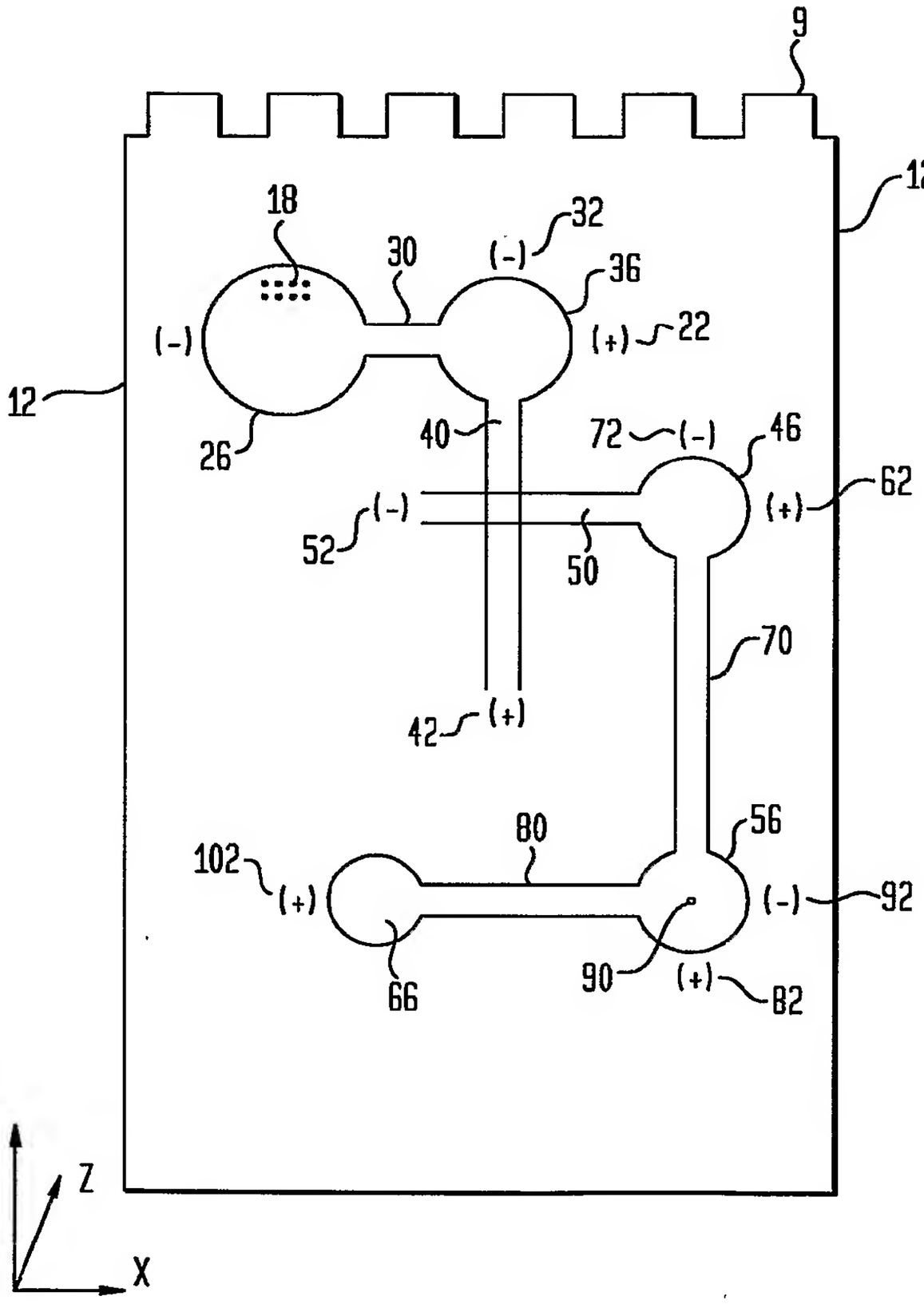
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FIG. 2